Vitamin D Receptor Polymorphism is Associated with Psoriasis

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Vitamin D receptor is a trans-acting transcriptional factor that mediates 1α,25-dihydroxyvitamin D_3 action in the regulation of target gene expression. Recent studies have shown that clinical response of psoriasis to 1α,25-dihydroxyvitamin D_3 is correlated with the vitamin D receptor mRNA expression level, which may be influenced by the genotype of the vitamin D receptor. In this study, we have explored a possible association between psoriasis and the polymorphism in the gene encoding the vitamin D receptor. We examined the allelic frequencies of the vitamin D receptor in psoriasis patients (n = 104) and in healthy controls (n = 104) by analyzing the restriction pattern of the polymerase chain reaction products. A significant increase in the frequency of the A allele (absence of the restriction site at intron 8) by *Apa*I restriction fragment length polymorphism was observed in psoriasis patients compared with that of the control group, and the tendency was more accentuated in early onset psoriasis. Odds ratios (95% confidence interval) for psoriasis of AA and Aa genotypes were 5.0 (1.3–19.1) and 2.4 (1.3–4.3), and odds ratios for early onset of AA and Aa genotypes were 6.4 (1.6–25.0) and 3.1 (1.7–5.9), respectively. Allele frequencies for A and a alleles were 0.317 and 0.683 in the psoriasis group and 0.168 and 0.832 in the control group (p = 0.001). A significant association between vitamin D receptor genotypes and the mean age at onset was observed (p < 0.05). Our findings suggest that allelic variance in the vitamin D receptor gene itself or other genes in linkage disequilibrium with this gene, could predispose to the development of psoriasis. *J Invest Dermatol 112:113–116, 1999*

Psoriasis is a common and persistent papulosquamous disease of unknown etiology, which affects up to 2% of the population (Krueger *et al*, 1984). It is characterized by hyperproliferation of the keratinocytes and inflammation. The therapeutic efficacy of 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] and its analogs has been tested and proved to be effective for the treatment of psoriasis (Kragballe *et al*, 1991). 1,25$(OH)_2D_3$ is the endogenously produced, hormonally active form of vitamin D_3. In addition to the known effect of 1,25$(OH)_2D_3$ on controlling calcium and bone metabolism (Reichel *et al*, 1989), it inhibits proliferation and induces terminal differentiation of cultured human keratinocytes (Smith *et al*, 1986), and can also modulate the immune system in a variety of ways. 1,25$(OH)_2D_3$ elicits its action on target tissues through the vitamin D receptor (VDR). The VDR is a member of the steroid/thyroid hormone receptor superfamily, which is a group of ligand-dependent transcription factors. The receptor-hormone complex binds to hormone response elements in regulatory regions of target genes, and modulates the gene transcription. It has been noted, however, that cultured fibroblasts and keratinocytes from some psoriatic patients have partial resistance to 1,25$(OH)_2D_3$ mediated anti-proliferative activity (MacLaughlin *et al*, 1985; Smith *et al*, 1988). Furthermore, clinical response to 1,25$(OH)_2D_3$ treatment is variable in patients with psoriasis. It has been shown that the intracellular level of VDR protein correlates with the cellular response to 1,25$(OH)_2D_3$ in cultured human colon cancer cell line (Zhao and Feldman, 1993), and altered induction of VDR mRNA in the treated psoriatic plaques is a marker for clinical responsiveness to 1,25$(OH)_2D_3$ treatment (Chen *et al*, 1996).

Recently, it has been reported that allelic variations of the VDR gene are associated with the risk of developing prostate cancer in men and osteoporosis in post-menopausal women (Morrison *et al*, 1994; Taylor *et al*, 1996). The polymorphism of the VDR gene can predict the differences in bone density, accounting for up to 75% of the total genetic effect on bone density in healthy individuals (Morrison *et al*, 1994). Although it was suggested that cDNA differences in the 3′ untranslated region of each VDR genotype may alter VDR mRNA levels (Morrison *et al*, 1994), the molecular mechanisms by which bone density is regulated by the VDR gene are not fully understood. The 3′ untranslated region polymorphisms are in strong linkage disequilibrium with restriction fragment length polymorphisms (RFLP) located in intron 8 (*Bsm*I and *Apa*I) and exon 9 (*Taq*I). Conflicting results have ensued concerning the association of the *Bsm*I RFLP in the VDR gene and bone density (Eisman, 1995). Despite the controversy, the physiologic parameters that are regulated by 1,25$(OH)_2D_3$, such as serum osteocalcin level and calcium absorption, are found to be correlated with VDR genotype (Morrison *et al*, 1992; Dawson-Hughes *et al*, 1995). These results suggest that genetic polymorphism of the VDR gene may influence 1,25$(OH)_2D_3$ mediated normal physiologic response of keratinocytes and can explain the variable responsiveness.

Although an association between VDR genotype and clinical response to 1,25$(OH)_2D_3$ or its analogs is not yet clear (Holick

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Abbreviations: 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; VDR, vitamin D receptor.

et al, 1996; Kontula *et al*, 1997), the variable inducibility or stability of VDR mRNA by $1,25(OH)_2D_3$ may reflect the heterogeneity in VDR genotype. Thus, the complexity of VDR genotype and variable responsiveness to $1,25(OH)_2D_3$ treatment provide a basis to test whether VDR gene may be one of the susceptibility genes for psoriasis. Recent studies have found a strong genetic background for psoriasis (Elder *et al*, 1994a). Although genome-wide searches for linked genes in affected families have come up with several chromosome regions cosegregating with the disease, the strongest association has been established for genes of the major histocompatibility complex or other related genes (Hoehler *et al*, 1997). Psoriasis shows a polygenic or multifactorial pattern of inheritance, the latter term distinguished by the additional involvement of environmental factors. The high heritability of the disease and studies indicating that susceptibility alleles can be inherited from parents with no personal or family history of psoriasis, lends support to the contention that one or more additional genes, not necessarily linked to the HLA locus, is a determinant of psoriasis susceptibility (Elder *et al*, 1994a; Henseler, 1997).

In this study, we report that the VDR polymorphism is associated with psoriasis by comparing the allele frequencies of VDR genotypes in psoriasis patients with those of normal healthy controls.

MATERIALS AND METHODS

Patients One hundred and four unrelated psoriasis patients, 52 men and 52 women aged 8–73 y (mean age, 37.1 ± 15.3 y), were recruited randomly from the Department of Dermatology, Seoul National University Hospital, for this study. All patients had psoriasis vulgaris with a duration of 0.1–41 y (mean duration, 12.2 ± 9.7 y). The age at onset of psoriasis ranged from 5 to 71 y old (mean age at onset, 24.9 ± 14.6 y). The patient group included 86 patients with early onset (onset not later than at the age of 40 y). All the patients were clinically evaluated concerning their family history of psoriasis, nail involvement, psoriatic arthropathy, and psoriasis area and severity index score. The normal control population consisted of 104 unrelated, healthy persons. All control subjects and psoriasis patients enrolled in this study had ethnic Korean background.

VDR genotyping The genomic DNA was extracted from leukocytes using standard methods (Sambrook *et al*, 1989). The VDR gene was amplified by using hemi-nested polymerase chain reaction (PCR). For detection of *Apa*I and *Taq*I sites, primer 1 (5′-CAGAGCATGGA-CAGGGAGCAAG-3′) in intron 8, and primer 2 (5′-GCAACTCCT-CATGGCTGAGGTCTCA-3′) and primer 3 (5′-AGGGGTTAGG-TTGGACAGGAGAGAG-3′) in exon 9 were used. For detection of *Bsm*I site, primer 1 (5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′) in exon 7, primer 2 (5′-TGGCGGCAGCGGATGTACGTCTGC-3′) in exon 9, and primer 3 (5′-AACCAGCGGGAAGAGGTCAAGGG-3′) in intron 8 were used (Morrison *et al*, 1994).

First PCR product was obtained using primers 1 and 2 with genomic DNA. Second PCR product was obtained using primers 1 and 3 with first PCR product. Each sample was subjected to 35 cycles in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT). After organic extraction of the second PCR product, the products were digested with respective restriction enzymes (New England Biolabs, Beverly, MA) according to the manufacturer's instructions. The digested samples were size fractionated by electrophoresis in a 1% agarose gel. Visualization after ethidium bromide staining was performed by means of ultraviolet fluorescence. Gels were photographed using a Polaroid MP-4 camera (Clifton, NJ) and analyzed for RFLP. The RFLP were coded as Aa (*Apa*I), Tt (*Taq*I), or Bb (*Bsm*I), where a uppercase letter signifies absence of the site and a lowercase letter signifies presence of the site. DNA analysis was performed blind to clinical data.

Statistical analysis Differences in the VDR genotypes were compared between psoriasis patients and controls by chi square test. Differences in the VDR genotypes were compared between early onset psoriasis patients and controls by Fisher's exact test. The VDR allele frequencies were deduced from genotype frequencies on the basis of Hardy–Weinberg equilibrium, and the case-control differences in the allele frequencies were also statistically analyzed with chi square test. Odds ratios were calculated with 95% confidence intervals. One-way analysis of variance test was used to compare group means of age at onset in the AA, Aa, and aa genotype groups. Chi square test was performed in the analysis of associations between other clinical variables and VDR genotypes, and in the analysis of sexual difference in VDR genotypes. The p value less than 0.05 was regarded as statistically significant.

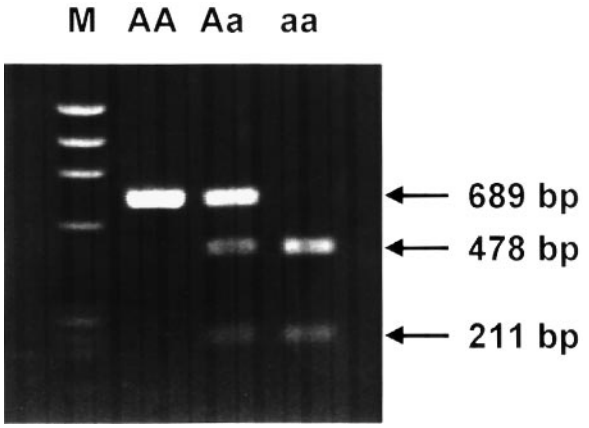

Figure 1. *Apa*I RFLP of the VDR gene. Genomic DNA was amplified using hemi-nested PCR method. The 689 bp second PCR product was digested with *Apa*I enzyme and size fractionated by 1% agarose gel electrophoresis. AA or aa denote the homozygote for the absence or presence of the *Apa*I site, respectively. Aa denotes the heterozygote. M, DNA size marker, ϕ174 *Hae*III digest.

Table I. Distribution of the VDR *Apa*I genotypes among psoriasis patients and controls, and odds ratios for psoriasis

Genotype	Controls (n = 104)[a]	All psoriasis (n = 104)	Early onset[c] (n = 86)
Frequency			
AA	3 (2.9%)	10 (9.6%)	9 (10.5%)
Aa	29 (27.9%)	46 (44.2%)	43 (50.0%)
aa	72 (69.2%)	48 (46.2%)	34 (39.5%)
Odds ratio [95% CI][b]			
AA/aa	1.0	5.0 [1.3–19.1]	6.4 [1.6–25.0]
Aa/aa	1.0	2.4 [1.3–4.3]	3.1 [1.7–5.9]

[a]n, number of investigated subjects.
[b]CI, confidence interval.
[c]Onset not later than at the age of 40 y.

RESULTS

Psoriasis patients showed significantly different *ApaI* RFLP VDR genotypes and allele frequencies 740 bp and 1850 bp first PCR product were obtained using primers 1 and 2 for *Apa*I and *Taq*I sites, and for the *Bsm*I site, respectively. Then, 689 bp and 825 bp second PCR products were obtained using primers 1 and 3, respectively. In each subject, the VDR genotypes were identified after restriction enzyme digestion of the second PCR product. In the psoriasis group, the frequencies for the BB, Bb, and bb genotypes were 1.8%, 5.5%, and 92.7%, respectively, and the frequencies for the TT, Tt, and tt genotypes were 94.5%, 5.5%, and 0.0%, respectively. Compared with the control group, no significant differences were observed for *Bsm*I and *Taq*I RFLP genotype frequencies.

In contrast, the significant difference in frequencies for *Apa*I RFLP genotype was observed between the psoriasis group and the control group. Digestion of the PCR segment with *Apa*I resulted in three genotypes: in the AA type only the 689 bp band was present; the Aa type included 689, 478, and 211 bp bands; and the aa type included 478 and 211 bp bands (**Fig 1**). Frequencies for AA, Aa, and aa genotypes were 10 (9.6%), 46 (44.2%), and 48 (46.2%) in the psoriasis group, and three (2.9%), 29 (27.9%), and 72 (69.2%) in the control group, respectively. We found a significant increase in subjects carrying AA and Aa genotypes in psoriasis patients (**Table I**). In addition, this tendency was more accentuated in the psoriasis patients with early onset. Odds ratios (95%

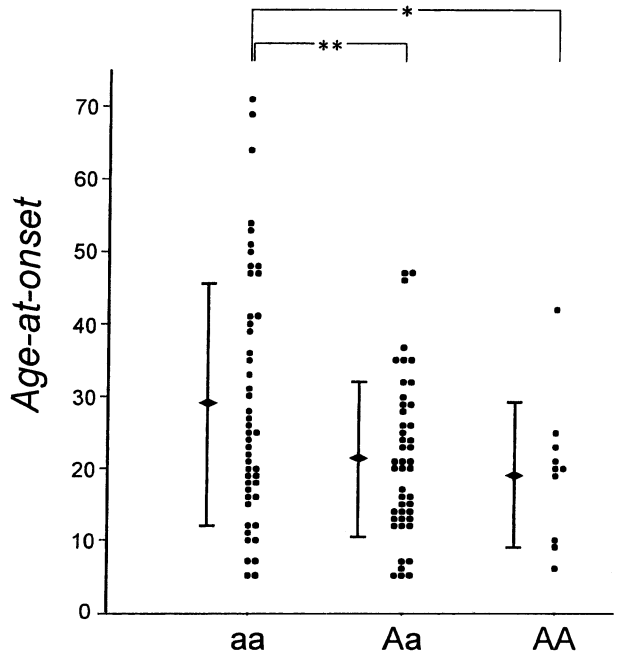

Figure 2. Age at onset and *Apa*I RFLP of the VDR gene. The distributions of age at onset in individuals with aa (n = 48), Aa (n = 46), and AA (n = 10) genotypes in psoriasis patients. Solid vertical bars indicate mean ± SD for each group (*p = 0.05, **p < 0.01).

Table II. Allele frequencies in controls and psoriasis patients

	Allele frequency		Odds ratio	
	A	a	[95% CI][b]	p
All psoriasis (n = 104)[a]	0.317	0.683	2.3 [1.4–3.7]	0.001
Early onset (n = 86)[c]	0.354	0.645	2.7 [1.7–4.4]	<0.001
Controls (n = 104)	0.168	0.832	1.0	

[a]n, number of investigated subjects.
[b]CI, confidence interval.
[c]Onset not later than at the age of 40 y.

confidence interval) for psoriasis of AA and Aa genotypes were 5.0 (1.3–19.1) and 2.4 (1.3–4.3), respectively. Odds ratios (95% confidence interval) for early onset of AA and Aa genotypes were 6.4 (1.6–25.0) and 3.1 (1.7–5.9), respectively. Derived allele frequencies for A and a alleles were 0.317 and 0.683 in the psoriasis group and 0.168 and 0.832 in the control group (**Table II**). Allele frequency for carrying A increased to 0.354 in the early onset psoriasis group. Haplotype analysis of *Bsm*I, *Taq*I, and *Apa*I RFLP was not possible due to absence of parental genotypes in this study.

No sexual difference in VDR genotype We have evaluated whether there is any sexual difference in the VDR genotypes. The frequencies for AA, Aa, and aa genotypes were seven (13.5%), 21 (40.4%), and 24 (46.1%) in the 52 male psoriasis patients, and three (5.8%), 25 (48.1%), and 24 (46.1%) in the 52 female psoriasis patients, respectively. There was no significant difference in VDR genotypes between male and female psoriasis patients.

Significant correlation between age at onset and VDR genoypes Means of age at onset, defined by the VDR polymorphism in the psoriasis patients, were compared among the three groups. Mean age at onset was 29.3 ± 17.2 (n = 48), 21.5 ± 11.1 (n = 46), and 19.1 ± 10.2 (n = 10) y old for homozygotes aa, heterozygotes Aa, and homozygotes AA, respectively, showing a significant relationship between VDR polymorphism and age at onset of psoriasis ($p < 0.05$) (**Fig 2**); however, other clinical variables such as family history of psoriasis, nail involvement, psoriatic arthropathy, and psoriasis area and severity index score, did not show any relationship with the VDR polymorphism (data not shown).

DISCUSSION

This study shows that there is a significant difference in allele frequencies and VDR genotypes between normal controls and psoriasis patients, especially with early onset. This suggests that allelic variance in VDR or genes in linkage disequilibrium with the VDR gene, may be a risk factor for development of psoriasis. Epidemiologic and clinical studies have shown that the peak age of onset for psoriasis is bimodally distributed. Early onset psoriasis is associated with a more severe and recurrent course, and increased inheritability in Caucasians (Henseler and Christophers, 1985). This tendency also applies to Koreans (manuscript in preparation). In this study, the more increased odds ratio of having AA or Aa alleles in the early onset group, and the significant difference in the mean age at onset among the three VDR genotype groups, underlies the significance of VDR polymorphism. Dominant genetic effect of carrying allele A was suggested by the excess of heterozygotes (Thomson and Bodmer, 1977). Kontula *et al* (1997) have previously shown that there is no difference in allelic variation of the *Bsm*I site in intron 8 of the VDR gene between psoriasis patients and controls. The apparent discrepancy between their results and ours may be explained by the fact that the size of the population was smaller, and that vitamin D responsiveness, rather than presence or absence of disease, was investigated in that study. Also, a different restriction site, e.g., *Bsm*I genotype, was examined in that study. Allelic variances in interleukin-1 receptor antagonist gene and tumor necrosis factor-α gene, as well as numerous HLA loci, were reported to be associated with psoriasis (Hoehler *et al*, 1997; Tarlow *et al*, 1997). Based upon the current clinical and genetic knowledge, psoriasis patients may show heterogeneous genetic make up (Elder *et al*, 1994b; Ortonne, 1996). Lack of absolute difference in VDR genotypes between psoriasis and normal controls further supports the heterogeneity of cause. There may be several other factors contributing to these relative differences: e.g., interaction of the VDR gene alleles with the environment or with other genes. Linkage disequilibrium to a nearby gene could also explain the lack of absolute difference between cases and controls.

It is unknown whether psoriasis is related with an intrinsic abnormality of the vitamin D_3 signaling pathway, and vitamin D_3 analogs improve psoriasis by overcoming an intrinsic abnormality of the vitamin D_3 signaling pathway in psoriatic skin (Kragballe, 1997). As the VDR mRNA levels may be influenced by the allelic variance of VDR (Morrison *et al*, 1994), the allelic differences between psoriasis and normal controls in this study may suggest the differences in the VDR mRNA levels; however, the VDR mRNA and protein levels were found to be similar between normal skin and involved and uninvolved psoriatic skin (Sølvsten *et al*, 1996). These results indicate that the quantity of the VDR mRNA or protein may not be the only explanation for variable responsiveness to $1,25(OH)_2D_3$ or the role of the VDR gene in the pathogenesis of psoriasis. The other possible explanations could be obtained from the studies of functional difference of VDR in relation to VDR genotype. These include interaction with other receptors and affinity for the target gene(s) that especially modulate the immune function.

The observed associations, which are not quite as strong, however, suggest another possible interpretation. Because association studies test the correlated occurrence of disease and an allele in a population, the population characteristics, such as population admixture in an ancient Korean population, could result in positive association of VDR polymorphism with psoriasis (Lander and Schork, 1994). This spurious association could arise if psoriasis-causing genes as well as allele A of the VDR gene happen to be more common in the tested Korean population. Therefore, further studies on this association, such as a transmission disequilibrium test, will be required to exclude this possibility (Spielman *et al*, 1993).

In conclusion, we report an association of a polymorphism in the VDR gene with psoriasis. This suggests that the VDR gene can be one of some candidate genes implicated in the pathogenesis of psoriasis in the Korean population. We cannot completely rule out the possibility that the association could represent population stratification, because a transmission disequilibrium test was not performed. It has already been revealed that there is a marked ethnic difference in the VDR genotypes (Tokita *et al*, 1996). It remains to be elucidated whether this polymorphism in the pathogenesis of psoriasis is also applicable to other ethnic groups. Further explanation of the molecular biologic or physiologic mechanism upon the basis of the VDR genotypes should be investigated.

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